Platelet adhesion and aggregation: Cell-resolved simulations and In vitro experiments

Van Rooij, B.J.M[1], Azizi, V.A.[1], Závodszky, G.[1], Ku, D.N.[2], Hoekstra, A.G.[1]

[1]Computational Science Lab, Institute for Informatics, Faculty of Science, University of Amsterdam, Amsterdam, The Netherlands

[2]Biofluids and Mechanical Device Research Group, Mechanical Engineering, Georgia Institute of Technology, Atlanta, USA

1. Introduction

High shear thrombosis happens on a thrombogenic surface (e.g. collagen) in a high shear environment and in presence of platelets and von Willebrand factor (vWF). In literature a discussion is going on between the need of an area with high shear [1] or an area with a high shear gradient [2]. However, the in vitro experiments used in these studies are performed in different flow chambers. In those flow chambers the specific flow fields are not the same. Currently, it is not known what specific flow characteristics cause high shear thrombosis. It is known that unfolding of the von Willebrand factor is regulated by elongational flow. However, this does not explain the formation of the platelet aggregates at the apex (high shear) or just behind the apex (high shear rate gradient), where vWF would contract instead of uncoil in the flow direction. However, note that the flow behaviour on the cellular level is hardly known and could provide additional insight [3].

Furthermore, we studied [3] the influence of the cell depleted layer on the start of a platelet aggregate. We applied our cell-based model on two in vitro experiments from literature to find in which area a platelet aggregate is favourable to start to from. We hypothesised that in a region of high shear rate in combination with a red blood cell-depleted layer with sufficient platelet flux could be an indicator of the start of a platelet aggregate. We observed that the platelet density was high in the area were the platelet aggregate formed.

In this study, we test this hypothesis by performing in vitro experiments and cell-based simulations to find out in what kind of fluid environment an aggregate starts to form and if the cell-free layer can predict where a platelet aggregate starts to form. To have a difference in cell-free layer in our experiments; we perform experiments with whole blood and platelet rich plasma. In the experiments with whole blood a cell-free layer is present and in the experiments with platelet-rich plasma a cell-free layer is absent due to the lack of red blood cells.

2. Material & Methods

In vitro experiments – In this study two microfluidic flow chambers are used. Firstly, a flow chamber designed by Casa et al. [4] based on an atherosclerotic stenosis with a contraction/expansion angle of 15 degrees and a stenosis length of 1mm. Secondly, a new designed flow chamber with a shorter stenosis (150 μm) and contraction/expansion angle 80 degrees (see Fig. 1). The microfluidic chips are made from PDMS, with a micromachined mold, plasma bonded on a microscopic glass plate. The channels on the microfluidic chips are coated with collagen Type I and the flow through the channel is regulated by a constant hydrostatic pressure (Δh). The experiments are performed with whole porcine blood (WB) and

platelet-rich plasma (PRP). The pressure heads are kept the same for WB and PRP. The out flow is measured with a mass balance. The clotting process is followed with a bright field microscope (DM6000B, Leica Microsystems, Germany) with a magnification of 5x and images are saved twice a second with a camera (Pixelfly, PCO, Germany). From the mass balance information, the occlusion time is calculated. The images are analysed to find the location where a platelet aggregation starts to form and where it occludes the microfluidic channel.

Cell-based simulations - The in vitro experiments are simulated with HemoCell, a validated cell-based blood flow model [5]. The shear stresses are measured at the places where a platelet aggregated was formed. Additionally, Newtonian and non-Newtonian fluid simulations are performed to compare the results with the cell-based simulations.

HemoCell is extended with a solidify function. This function solidifies a platelet if it feels a shear rate that is high enough to unfold vWF and if it is close enough to a binding site. A binding site is a boundary specified with a bounding box or a platelet that already has been solidified. The probability to bind is the multiplication of those two factors.

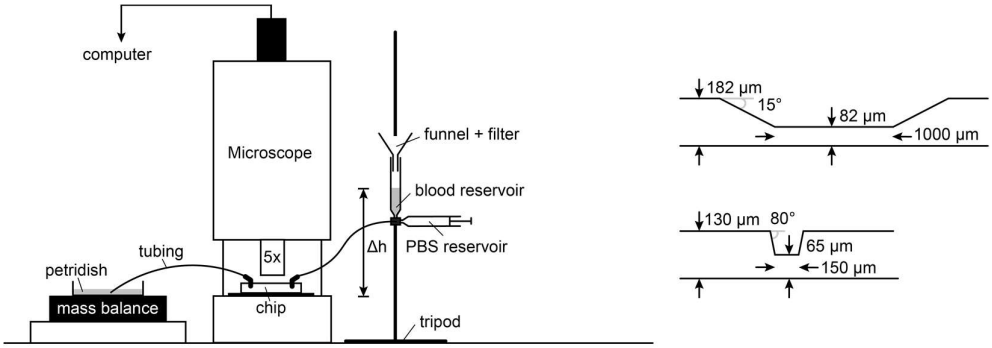

Figure 1 *The set-up of the in vitro experiment is shown on the left side and the side view of the used chips is shown on the right side of the image. The depth of both chips is 480 µm.*

3. Results

The in vitro experiments give more inside in where a platelet aggregate starts to form. In figure 2 an example of an experiment in the new designed chip with PRP (left) and WB (right) is shown. In both situations the platelet aggregate obstructs the microfluidic channel in the stenosis area, which is easier to observe in the PRP experiment. This was as well the case for Casa's chip. In Casa's chip we observed that the obstruction of the channel was in the upstream part of the stenosis. The occlusion times, for both PRP and WB, where higher for experiments performed at high shear rates. When PRP is compared to WB, the occlusion times are more than twice as high.

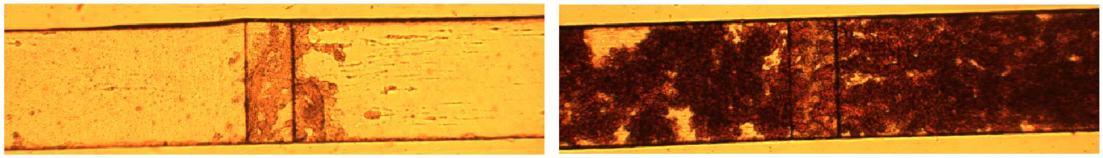

Figure 2 *The last frame of an in vitro experiment with the 80 degrees angle chip design are shown. On the left an experiment with platelet rich plasma is shown and on the right an experiment with whole blood. In both cases the platelet aggregate is formed in the stenosed area.*

The cell-based simulations showed an elevated shear rate in the stenosed areas for both microfluidic chips. In the new chip a recirculation zone was found behind the stenosis section. The shear rates varied from 6000 s^{-1} till 20,000 s^{-1}. In the PRP experiments higher shear rates were obtained by the same pressure gradient due to the decrease in viscosity of blood plasma versus blood. We calculated as well the platelet densities in the cell-based simulations. The platelet densities where lower in the stenosed section in the case of PRP in comparison to WB. The solidify extension is tested in this study. Preliminary results show that the initial platelet adhesion can take place anywhere at the high shear rate zone, this is due to the set-up of the code. The code has to be tested more and the validation using the experiments described is one of the next steps.

4. Discussion

One of the main results is that the occluding clot formed in a high shear rate area, both for the platelet-rich plasma and whole blood. Additionally, an increase in occlusion time is observed when the shear rate increased. And whole blood occluded twice as fast as platelet-rich plasma. From the simulation we found an increased platelet flux in the experiments with whole blood. The shear rates in all experiments where high enough to uncoil von Willebrand factor and form platelet aggregates. The platelet aggregates start to form anywhere in the stenosed section; however, occlusion took place in the upstream part when Casa's chip was used. A reason for this could be a high shear rate and a high platelet flux at the entrance of the stenosed section. The PRP and WB showed a different occlusion time. In whole blood, red blood cells are present and collisions between red blood cells and platelets results in margination. More platelets will be present in the cell-free layer. In comparison to the platelet-rich plasma experiments where no cell-free layer is formed. This results in a lower platelet flux for the PRP experiments and that could be a reason for the delay in occlusion time. This tells us that we do not need red blood cells to form a platelet aggregate, however, they are necessary to make the process faster. In haemostasis there is a balance between bleeding and clotting, so red blood cells could shift these two processes in a way that there is a healthy balance.

The platelet solidification model is a preliminary version of a cell-based thrombosis model what we are working on. The model is able to let platelets bind in areas where von Willebrand factor is available in its uncoiled form. The modelled showed an initial platelet aggregate formation at the stenosed section. The model will be extended with an advection-diffusion model for the activation of platelets.

References

[1] Dongjune A. K., Casa, L. D. C. & Ku D. N. (2018). Arterial thrombosis depends on shear rate more than shear rate gradient. *Poster ISTH SSC 2018.*

[2] Tovar-Lopez, F. J., Rosengarten, G., Westein, E., Khoshmanesh K., Jackson, S.P. Mitchell, A. & Nesbitt, W. S. (2010). A microfluidics device to monitor platelet aggregation

dynamics in response to strain rate micro-gradients in flowing blood. *Lab on a Chip.* 10(3):291-302.
[3] van Rooij, B. J. M., Azizi, V.A., Závodszky, G., & Hoekstra, A.G. (Submitted, 2018) Identifying the start of a platelet aggregate by the shear rate and the cell depleted layer. *Journal of Royal Society Interface.*
[4] Casa, L. D. C., Gillespie, S. E., Meeks, S. L., & Ku, D. N. (2016). Relative contributions of von Willebrand factor and platelets in high shear thrombosis. *Journal of Hematology & Thromboembolic Diseases.*
[5] Závodszky, G., Van Rooij, B., Azizi, V., Hoekstra, A. (2017), Cellular Level In-silico Modeling of Blood Rheology with An Improved Material Model for Red Blood Cells. *Frontiers in Physiology*, 8, 563